

Signalling between G-protein-coupled receptors and TASK1 channels

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This editorial refers to ‘Inhibition of the potassium channel TASK-1 in rat cardiac muscle by endothelin-1 is mediated by phospholipase C’ by J. Schiek et al., pp. 97–105, this issue.

The superfamily of two pore-domain K⁺ (K_{2P}) channels has at least 15 members,¹ of which mainly TASK1 is expressed in cardiac myocytes. TASK1 gating is voltage independent, but the channel is acid sensitive, being activated by alkalotic conditions. Ion currents carried by TASK1 likely contribute to the cardiac action potential duration (APD), and pharmacological inhibition of TASK1 lengthens the APD^{2,3} while TASK1 knock-out animals display a long QT on the electrocardiogram.^{4,5} Interest in TASK1 channels has been mounting, because their activity in various cell types seems critical for several physiological processes while their changed expression and/or function may underlie many disease conditions. The channels are potential targets for diverse therapeutic actions, including antiarrhythmic, antihypertensive, antiepileptic, and antidepressive treatments as well as analgesia and inhalation anaesthesia.⁶ Therefore, identifying the signalling pathways involved in their regulation is important. Newly available investigation tools, such as those used in the study of Schiek et al.⁷ published in the current issue of *Cardiovascular Research*, allow one to investigate the signalling pathways for the regulation of TASK1 in intact cells while monitoring cell membrane lipid metabolism.

A well-recognized regulation of TASK1 channels is their inhibition by receptors coupled to the G-protein G_p, which is linked to membrane phospholipid breakdown via the activation of phospholipase C (PLC). Neurotransmitters such as norepinephrine (acting on α-adrenergic receptors), hormones such as angiotensin or endothelin-1, and other agonists (e.g. platelet-activating factor, PAF) reduce TASK1 ion currents.¹ The signalling pathway linking G_p activation to TASK1 inhibition has been a source of controversy, given variable and inconsistent results obtained in different cell systems. Some studies have suggested a role of TASK1 phosphorylation by protein kinase C (PKC) while others did not find any evidence for involvement of the kinase. Similarly, some studies have provided evidence for involvement of phosphatidylinositol biphosphate (PIP₂) breakdown while others obtained evidence for the opposite. Nearly all possible steps, including a direct TASK1

inhibition by G_p subunits, have found supporting and opposing evidence. Such variability in the results may simply reflect the diversity of PLC and PKC isozymes and of other possible transducers and effectors involved in the various cells.

Identifying the signalling pathways for cardiac endothelin-1 effects is important given the increased circulating and intramyocardial levels of the peptide in conditions such as hypertrophy, heart failure, and acute ischaemia. Using rat ventricular myocytes, Schiek et al.⁷ demonstrate that endothelin-1-mediated inhibition of sustained K⁺ currents in cardiac cells occurs via subtype-A receptors and involves an effect on TASK1. The study further explores the signalling pathways and provides results that challenge paradigms (e.g. the involvement of PKC) that are accepted for other cell types or for other cardiac G_q-coupled receptors. PLC is implicated given the suppression of the endothelin-1 effect following a pretreatment with the PLC inhibitor U73122. The effects can be reconstituted in a heterologous expression system, where endothelin-1-induced breakdown of PIP₂ as a result of PLC activation can be readily demonstrated. This study shows that the mechanisms involved in the inhibition of cardiac TASK1 lie downstream of the G_p subunits, implicated as acting directly on TASK1 in other systems. Having established an involvement of PLC, three possible signalling pathways downstream of the enzyme can be invoked: (i) the accumulation of IP₃, (ii) the accumulation of diacylglycerol (DAG), or (iii) the depletion of PIP₂. While IP₃ usually acts by releasing Ca²⁺ from intracellular stores, and DAG by activating PKC, they may also act directly on channels. Indirect IP₃-mediated regulation of TASK1 via Ca²⁺ release is unlikely because the regulation of the channels is insensitive to a blockade of Ca²⁺ release from the sarcoplasmic reticulum.⁸ In addition, although IP₃-induced Ca²⁺ release can occur in cardiac cells, it is usually of minor significance compared with Ca²⁺-induced Ca²⁺ release. Also, direct IP₃ injection in oocytes failed to inhibit TASK1.⁸ Concerning DAG, indirect action via PKC_ε is involved in the PAF-induced inhibition of TASK1 and its resultant arrhythmogenic effect in mouse ventricular cells since these effects could be reproduced by PKC activators and prevented by PKC inhibitors.⁹ However, the PKC_ε phosphorylation site is located in a non-conserved region of TASK1, leaving the possibility that TASK1 in cardiomyocytes of other species may respond differently. In the study of Schiek et al.⁷ an involvement of PKC is

excluded in the endothelin-1 effects, which were insensitive to PKC inhibitors.

Having ruled out roles for IP_3 and PKC in the endothelin-1 effect on cardiac TASK1, one is apparently left with the depletion of PIP_2 as the remaining hypothesis. This hypothesis is very attractive given the reported role of PIP_2 as a major channel regulator. Requirement of PIP_2 for channel gating has been proposed when manipulations that likely deplete PIP_2 cause channel closure or rundown, whereas manoeuvres that replenish PIP_2 restore or allow sustained channel activity. In cardiac cells, requirement of PIP_2 has been proposed for various K^+ channels, including K_{V7} -KCNE1 (I_{Ks}), $K_{ir2.1}$ (I_{K1}), I_{K-ACH} , and I_{KATP} channels as well as for other channels. However, it is being increasingly recognized that the ability of exogenous PIP_2 to restore channel activity may not be a sufficient proof for a PIP_2 regulation of these channels under physiological conditions. Indeed, many channels reported to be PIP_2 sensitive in experiments on isolated membrane patches do not respond to protocols of PIP_2 depletion in intact cells, suggesting that non-physiologically high PIP_2 concentrations used experimentally may form micelles and/or may interact with channel domains that are not affected by physiological levels of the phospholipid.¹⁰ PLC activation by physiological factors may not be sufficient to reduce PIP_2 to levels below those needed for channel activation, especially under conditions where synthesis of PIP_2 by lipid kinases remain active and/or when the channel displays high affinity for PIP_2 . Using novel protocols of PIP_2 depletion from the cell membrane, it was recently shown that the TASK1 activity in CHO cells is unaffected by the lipid depletion,¹¹ making unsustainable the

apparently remaining hypothesis of PIP_2 depletion as a mediator of the effect of endothelin-1.

Obviously, other mechanisms have to be invoked for the endothelin-1 inhibition on cardiac TASK1, including (i) a direct action of PLC itself, (ii) a PKC-independent effect of DAG or, as suggested by Schiek et al.,⁷ (iii) an effect of DAG metabolites. Direct channel regulation by PLC is known to exist for TRPC3 through formation of an intermolecular lipid-binding domain.¹² Concerning a direct action of DAG itself, there are many examples of channel modulation by this lipid, including an inhibition of TREK, another K_2P channel.¹³ A possible direct effect of DAG on cardiac TASK1 has not been tested, but in carotid body cells, where the channel may be involved in detecting hypoxia, oleoyl-acetyl-glycerol, a DAG analogue, had no effect in the presence of PKC inhibitors, making it unlikely that the lipid can directly inhibit TASK1.¹⁴ Similarly, DAG failed to inhibit neuronal TASK channels.¹³ The only remaining possibility is that of an involvement of DAG metabolites. It is of interest to note that arachidonoyl-ethanolamide, also called anandamide, which can be derived from membrane phospholipids (Figure 1) by the action of a PLC,^{15,16} is an inhibitor of TASK1. Anandamide is an endogenous cannabinoid but its effects on TASK1 are independent of cannabinoid receptors. 2-Arachidonoyl-glycerol, another endogenous cannabinoid structurally very close to anandamide, can be produced from DAG by a DAG lipase (Figure 1), but its effects on TASK1 are unknown. Other possible metabolites include arachidonic acid and phosphatidic acid. Some members of the K_2P channel family are sensitive to arachidonic acid, which however stimulates rather than inhibits them as would be expected if the lipid were to mediate the effect of endothelin-1. Phosphatidic acid failed to inhibit neuronal TASK.¹³ Obviously, additional experiments using modulators of the enzymes involved in the formation/breakdown of DAG metabolites are needed in order to elucidate the signalling between G_p -coupled receptors and TASK1, but it is likely that the pathways are diverse, reflecting the diversity of enzymes and channels involved in each cell. Modulating the pathways implicated in the regulation of cardiac TASK1 could be of clinical relevance to prevent arrhythmogenic actions of endothelin-1 in conditions such as hypertrophy, heart failure, and acute myocardial ischaemia.¹⁷ In addition, activating TASK1 or opposing its inhibition by endothelin-1 may also contribute to reducing Ca^{2+} influx during the action potential, hence reducing the rise in intracellular Ca^{2+} , which likely plays a role in the remodeling process associated with hypertrophy.

Conflict of interest: none declared.

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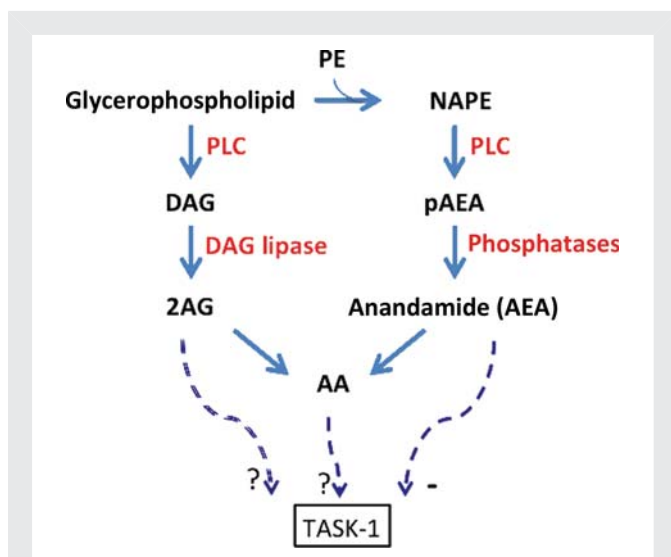


Figure 1 Possible pathways for PLC-dependent but PKC-independent inhibition of cardiac TASK1. The scheme shows the formation of anandamide and of 2-arachidonoyl-glycerol from membrane phospholipids using pathways involving phospholipase C isoforms^{15,16}; also shown are possible interactions of reaction products with TASK1. Solid arrows indicate biochemical transformations. Interrupted arrows indicate inhibitory (–) and unknown (?) interactions with TASK1. AA, arachidonic acid; AEA, arachidonoyl-ethanolamide; 2AG, 2-arachidonoyl-glycerol; DAG, diacylglycerol; NAPE, N-arachidonoyl-phosphatidylethanolamine; pAEA, phospho-arachidonoyl-ethanolamide; PE, phosphatidylethanolamine; PLC, phospholipase C.

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